

AD-A056 085 ALABAMA UNIV MEDICAL CENTER BIRMINGHAM
A STUDY OF ADVERSE GASTROINTESTINAL EFFECTS OF 8-AMINOQUINOLINE--ETC(U)
SEP 77 R S TEAGUE, R L MUNDY, E R SEIDEL DADA17-67-C-7136
NL

UNCLASSIFIED

| OF |
ADA
056085



END
DATE
FILED
B -78
DDC

AD A 056085

LEVEL

AD _____

12

A Study of Adverse Gastrointestinal Effects
of 8-Aminoquinolines

ANNUAL PROGRESS REPORT

For the period 1 May 1976 to 1 June 1977

by

Robert S. Teague, M.D., Ph.D.

Roy L. Mundy, Ph.D.

and

Edward R. Seidel, M.S.

Report Date September 1977

Supported by



U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Office of the Surgeon General, Fort Detrick, Frederick, Maryland 21701

Contract No. DADA 17-67-C-7136

AD No. _____
DDC FILE COPY

University of Alabama Medical Center,
Birmingham, Alabama 35294

Approved for public release: distribution unlimited

78 07 06 066

AD _____

(12)

A Study of Adverse Gastrointestinal Effects
of 8-Aminoquinolines

ANNUAL PROGRESS REPORT

For the period 1 May 1976 to 1 June 1977

by

Robert S. Teague, M.D., Ph.D.

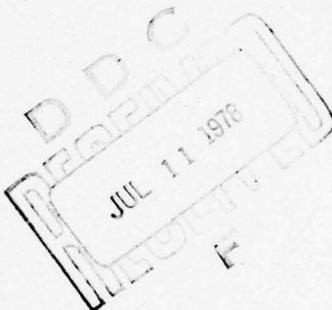
Roy L. Mundy, Ph.D.

and

Edward R. Seidel, M.S.

Report Date September 1977

Supported by



U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Office of the Surgeon General, Fort Detrick, Frederick, Maryland 21701

Contract No. DADA 17-67-C-7136

University of Alabama Medical Center,

Birmingham, Alabama 35294

Approved for public release: distribution unlimited

78 07 06 066

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) A Study of Adverse Gastrointestinal Effects of 8-Aminoquinolines		5. TYPE OF REPORT & PERIOD COVERED Annual Report 1 May 1976 - 1 June 1977
6. AUTHOR(s) Robert S. Teague, M. D., Ph. D. Roy L. Mundy, Ph. D. Edward R. Seidel, M.S.		7. CONTRACT OR GRANT NUMBER(s) DADA 17-67-C-7136
8. PERFORMING ORGANIZATION NAME AND ADDRESS University of Alabama Medical Center Birmingham, Alabama 35294		9. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62759A 10. 16 3A762759A829/00/024
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick Frederick, Maryland 21701		12. REPORT DATE 11 September 1977 NUMBER OF PAGES 54 pages
13. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) 12 55P		14. SECURITY CLASS. (of this report) Unclassified
15a. DECLASSIFICATION/DOWNGRADING SCHEDULE		
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Primaquine, Gastrointestinal Musculature, Type I Activity, Writhing Test, Antimuscarinic, Gall Bladder, Cholecystokinin, Adverse Effects.		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Investigations were carried out in an attempt to uncover the mechanism of primaquine-induced gastrointestinal discomfort reported in humans after oral administration of the compound. Primaquine did not alter recordings of gastrointestinal motor activity in unanesthetized rats or dogs. Primaquine acted as an analgesic in the mouse writhing test. It was reported that primaquine was a surmountable antagonist of acetylcholine in the isolated guinea pig gall bladder. (PA ₂ = 4.57). Cholecystokinin was shown to potentiate		

20. Continued

the action of acetylcholine in the isolated guinea pig gall bladder, WR 6890, a congener of primaquine (6-hydroxy-8-aminoquinoline), neither contracted nor relaxed the isolated guinea pig ileum or gall bladder and had no effect on the sensitivity of these organs to acetylcholine. The results of these experiments do not explain the mechanism of primaquine-induced distress.

94

ACCESSION for	
NTIS	<input checked="" type="checkbox"/> White Section <input type="checkbox"/>
DDC	<input type="checkbox"/> Bell Section <input checked="" type="checkbox"/>
UNANNOUNCED	<input checked="" type="checkbox"/>
JUSTIFICATION	
BY	
DISTRIBUTION/AVAILABILITY CODES	
DI	DLA
A	

TABLE OF CONTENTS

FOREWORD	2
SUMMARY	3
INTERIM REPORT No. 8, Lack of Effect of Primaquine on Type I Activity of the Duodenum of Unanesthetized Rats	4
INTERIM REPORT No. 9, Preliminary Report on the Use of Extraluminal Force Transducers to Study the Gastrointestinal Effects of Candidate Antimalarial Agents	12
INTERIM REPORT No. 10, Antagonist of Primaquine to Intraperitoneal Acetic Acid in Mouse Writhing Test	19
INTERIM REPORT No. 11, Antimuscarinic Activity of the 8-Aminoquinoline Antimalarial Drug, Primaquine, on the Gall Bladder of the Guinea Pig	25
INTERIM REPORT No. 12, Potentiation of the Effects of Acetylcholine and Oxotremorine on the Gall Bladder of the Guinea Pig by Cholecystokinin and the Interaction of Primaquine with Cholecystokinin	35
INTERIM REPORT No. 13, A Study of a Congener of Primaquine on the Isolated Gall Bladder and Ileum of the Guinea Pig.	46

FOREWORD

Period Covered: 1 May 1976 to 1 June 1977

Animal Experimentation: In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

Disposition Instructions: Unclassified report. When this document has served its purposes, DESTROY IT.

SUMMARY

This report contains six interim reports submitted to the Pharmacology Department, Walter Reed Army Institute of Research, concerning the mechanism of the adverse gastrointestinal effects of 8-aminoquinolines.

The report encompasses work completed during the period 1 May 1976 to 1 June 1977 and each study is summarized as follows:

8. Primaquine, when administered orally to two lightly restrained, unanesthetized rats with chronically implanted duodenal recording catheters, failed to produce a consistent alteration of Type I activity.

9. Acutely or chronically implanted extraluminal strain gauge transducers were used to study the effects of primaquine on the motor activity of the GI tract of the dog. In neither anesthetized nor unanesthetized animals did primaquine cause any consistent effects on the musculature of the stomach, duodenum, jejunum, ileum or proximal large bowel.

10. Primaquine diphosphate was injected i.p. in mice to see if it produced writhing like acetic acid or phenylquinone. Primaquine (10^{-3} M, 0.02 ml/gm) failed to produce writhing. When injected simultaneously with acetic acid, primaquine displayed analgesic activity attenuating acetic acid-induced writhing. The mechanism of this protective action was not explained.

11. The hypothesis that primaquine might cause epigastric pain by producing spasm of the gall bladder musculature was tested in the isolated gall bladder. The results did not support the thesis. In fact, primaquine was shown to be a weak competitive antagonist ($pA_2 = 4.57$) of acetylcholine in the preparation.

12. The interaction of primaquine and the C-terminal octapeptide of cholecystokinin was studied in the isolated gall bladder. Primaquine was shown to be a competitive antagonist of the peptide. The paper also reports a potentiating action of cholecystokinin on acetylcholine and oxotremorine in the preparation.

13. Two congeners of primaquine were studied in isolated smooth muscle preparations. The 6-hydroxy-8-aminoquinoline derivative (WR 6890) neither contracted nor relaxed the isolated ileum or gall bladder. The compound had no effect on the sensitivity of either tissue to cumulative doses of acetylcholine. The 6-methoxy-8-aminoquinoline derivative was insoluble in aqueous solvents and therefore inappropriate for use in the isolated organ preparation.



the University of Alabama in Birmingham / UNIVERSITY STATION / BIRMINGHAM, ALABAMA 35294

the Medical Center /DEPARTMENT OF PHARMACOLOGY /August 17, 1976

LACK OF EFFECT OF PRIMAQUINE ON TYPE I ACTIVITY
OF THE DUODENUM OF UNANESTHETIZED RATS

ROBERT S. TEAGUE

ROY L. MUNDY

Contract Number

DADA 17-67-C-7136

Interim Report Number 8

ABSTRACT

Primaquine, when administered orally to two lightly restrained, un-anesthetized rats with chronically implanted duodenal recording catheters, failed to produce a consistent alteration in Type I activity. Fifty or 100 mg/kg doses of primaquine diphosphate, PO, did not cause increased frequency of burst activity, duration of individual bursts or maximum intensity of the contractions in each burst.

Wide variations in control patterns and lack of a consistent drug-related change in waveforms makes results from this technique difficult to interpret. It is concluded that experiments using this system should be abandoned.

TO: Department of Pharmacology, Walter Reed Army Institute of Research,
Walter Reed Army Medical Center, Washington, D. C. 20012

SUBJECT: LACK OF EFFECT OF PRIMAQUINE ON TYPE I ACTIVITY OF THE DUODENUM OF
UNANESTHETIZED RATS

INTRODUCTION

Earlier reports from this laboratory had noted lack of a consistent effect of primaquine on gastrointestinal activity. Most of the experiments had been conducted on animals anesthetized with pentobarbital. The experiments reported here were carried out on conscious, lightly restrained rats.

The Handbook of Physiology (1) describes 2 basic types of normal motor activity in the small intestines. Type I activity (Fig. 1) refers to the simple, rapid, monophasic, low amplitude waveforms. This may be contrasted to the more complex, longer, multiphasic Type III activity. Experiments were carried out to determine whether primaquine, in an oral dose of 50 mg/kg body weight, had any effect on the frequency of occurrence, amplitude, or duration of Type I waveforms.

METHODS

Intraluminal, open-ended catheters were implanted in the duodenum of 2 male rats weighing 250 g each. They were anesthetized for surgery by the intraperitoneal injection of 50 mg/kg of pentobarbital sodium. A laparotomy was performed and an area of the duodenum, approximately 4 cm caudal to the pylorus located. A length of PE 100 polyethylene tubing was carried beneath the skin in a skin tunnel from the base of the dorsal neck to the abdominal incision. A purse-string suture anchored the tubing at the neck incision and a segment of tubing was allowed to protrude about two cm from the incision so that pressure transducers might be attached to it.

A trocar (16 hypodermic needle) was advanced through the wall of the duodenum. The free end of the PE tubing, tipped with a 2 cm length of silastic tubing was inserted through the trocar 1 cm into the lumen of the gut. The trocar was removed and a suture which had been passed through the duodenal wall was tied around the tubing. Two additional sutures were cemented (cyanoacrylate) to the catheter 2 cm from its entry into the duodenum. These two sutures were then sewn into the gut wall to keep the catheter in place. The peritoneum and skin were closed. Two weeks were allowed for recovery from surgery before drug treatment.

The rats were placed in plexiglass restraint cages and the end of the intraluminal catheter was attached to a Hewlett-Packard pressure transducer. Intraluminal pressure changes were recorded from this system on an eight channel oscillograph. A cotton cloth was draped over the restraint cage to further isolate the animal and a 30 minute accommodation period allowed prior to commencement of recording. A volume of 0.5 cc of Tyrode's solution was used, as needed, to flush the catheter. The rats were never kept in the restraint cage for more than 2 hours during a session and were returned to the colony cage for at least 30 minutes between recording sessions. Recording

experiments lasted from 3 to 10 hours.

Primaquine, as the diphosphate salt, was dissolved in deionized water and administered by gavage at a dose of either 50 or 100 mg/kg. Equal volumes of Tyrode's solution were injected in control experiments.

Records were analyzed for frequency of occurrence or the number of Type I bursts per hour, duration of each burst and the amplitude or maximum intensity of the individual components of a burst as measured in mmHg (Fig. 1). A composite activity score was calculated by multiplying the frequency of the Type I activity (bursts per hour) times the duration of the bursts (minutes).

RESULTS

Data obtained in each rat during several experiments are shown in Table 1. No effort was made to summarize or analyze the results further, since it can be seen from inspection that no consistent pattern of drug-related activity was observed.

RAT #1. At no time after drug treatment did the recorded parameters differ widely from the baseline control observations (days 7-14). On the fifteenth day post-operation 50 mg/kg of primaquine was given orally. No Type I activity was recorded before treatment and very little activity was observed after treatment. It is concluded that there was no drug effect. On the seventeenth day post-operation the rat received a second 50 mg/kg dose primaquine. At 130 minutes post-treatment the composite activity was less than during the control period for that day but the maximum intensity of the waveforms had increased. At 170 minutes this pattern had changed so that composite activity exceeded that of the control period and intensity was near normal. At 240 minutes both parameters had returned to control levels. The catheter became dislodged from the gut on the eighteenth day. Autopsy revealed that the catheter had been placed in the upper duodenum.

RAT #2. Sixty minutes after the first drug treatment in this animal, composite and maximum activity were attenuated. At 240 minutes these parameters exceeded control and at 540 minutes activity was less but intensity greater than during the control period. After the second drug treatment composite activity was slightly increased after 130 minutes and more than halved at 170 minutes. Intensity was attenuated 33% at 130 minutes and 23% at 170 minutes. During the control period prior to the 100 mg/kg dose of primaquine constant Type I activity was recorded for 60 minutes. After drug treatment, normal intermittent bursts of activity were observed. The composite activity score after this large dose was the lowest observed to that point.

The following day activity and intensity were normal but 48 hours after treatment another extremely low composite activity score was recorded. Autopsy showed that the recording catheter was located in the duodenum 2 cm from the pylorus.

DISCUSSION

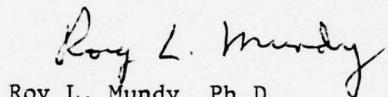
It has been difficult to find literature references to recordings of Type I activity in the conscious rat. However, recording of this waveform in the human

is commonly reported. Our recordings of these waveforms in the conscious, lightly restrained rat were similar qualitatively to those recorded from the human small bowel.

In our experiments we were unable to observe consistent changes in these waveforms following prim-quine treatment. There also appears to be large differences in the activity of the bowel of the rat with no treatment which would make it extremely difficult to interpret drug effects. For these reasons we believe that further experiments of this nature in the rat would be of little value.



Robert S. Teague, M. D., Ph.D.
Professor and Chairman



Roy L. Mundy, Ph.D.
Professor

FIGURE I

Example of a Type I burst recorded from an
indwelling, intraduodenal catheter.

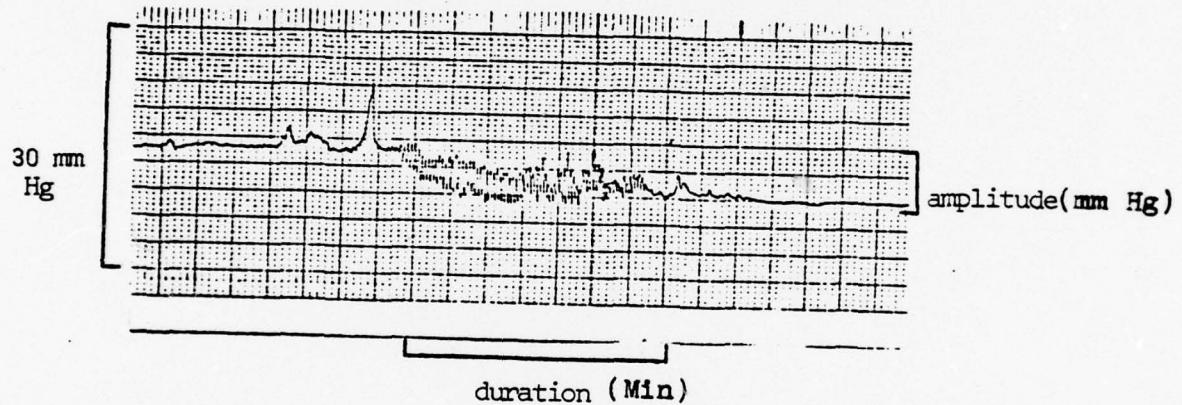


TABLE I
The composite activity and maximum intensity of Type I activity in the rat duodenum.

Primaquine (mg/kg; PO)	Day Post Operation	Minutes Post Treatment	RAT #1		RAT #2	
			Composite Activity*	Maximum Intensity (mm Hg)	Composite Activity*	Maximum Intensity (mm Hg)
7	control	22.5	14	0 +	18	15
	control	0				
8	control	12	22	50	24	22
	control	0				
14	control	12	22	50	18	17
	control	0				
15	control	0			24	22
	60	0				
50	240	24	19	44	18	5
	540	0				
16	control	10	26	22	9	15
	control	18	11	45		
17	130	10	20	52	22	11
	170	30	12	20		
50	240	21	11	0Δ	45	23
	control	--**	--			
100	21	170	--	--	15	> 30
	control	--	--			
22	control	--	--		50	15
	control	--	--			
23	control	--	--		7	19
	control	--	--			

* - Frequency (bursts per hour) X duration of bursts (in minutes)

** - Lost catheter

+ - No Type I activity but undifferentiated activity present

Δ - No Type I activity or undifferentiated activity

REFERENCES

1. Handbook of Physiology; Sec. 6, Vol IV. Motility; American Physiological Society, Washington, D. C., 1968.



the University of Alabama in Birmingham/ UNIVERSITY STATION / BIRMINGHAM, ALABAMA 35294

the Medical Center /DEPARTMENT OF PHARMACOLOGY /September 8, 1976

PRELIMINARY REPORT ON THE USE OF EXTRALUMINAL
FORCE TRANSDUCERS TO STUDY THE GASTROINTESTINAL
EFFECTS OF CANDIDATE ANTIMALARIAL AGENTS

ROBERT S. TEAGUE

ROY L. MUNDY

Contract Number

DADA 17-67-C-7136

Interim Report Number 9

ABSTRACT

The method of Jacoby (1) has been used to study the effect of primaquine diphosphate on circular muscle tone and activity in 6 anesthetized and 4 unanesthetized dogs. In the anesthetized dogs, primaquine was administered by mouth and in some instances applied directly to the serosal or mucosal surface of the gastrointestinal segment under study. All of the conscious dogs received the drug by mouth on one or more occasions at a dose of 10 or 20 mg/kg of the diphosphate salt. Studies were made of stomach, duodenum, ileum, jejunum and proximal large bowel.

The acutely or chronically implanted strain gauge appeared to record muscle activity faithfully as evidenced by: (a) producing tracings which were comparable to those of Jacoby, (b) reaction of the dogs to morphine sulfate (a known stimulator of intestinal tone) and (c) activity of the muscle during bouts of vomiting.

Gross pathology, consisting of minute sub-serosal hemorrhages, was noted in many of the chronic preparations. It is not known whether this pathology was drug related or brought about by the "stressful" nature of the experiments.

TO: Pharmacology Department, Walter Reed Army Institute of Research,
Walter Reed Army Medical Center, Washington, D. C. 20012

SUBJECT: Preliminary report on the use of extraluminal force transducers
to study the gastrointestinal effects of candidate antimalarial
agents.

INTRODUCTION

Experience in our laboratory has indicated that spontaneous gastrointestinal activity is affected by anesthesia. Previous reports have also indicated that primaquine might affect gastrointestinal tone or motility by stimulation of reflexes mediated through the central nervous system. Our research on changes in gastrointestinal function after primaquine treatment would therefore be most appropriately measured in conscious animals. The method of Jacoby (1) for the measurement of contractility in conscious dogs with extraluminal strain was adapted for use in the experiments reported here.

In order to gain experience in the use of the technique, a series of six dogs were studied under pentobarbital anesthesia. This allowed us to learn about attachment of the transducers and the proper operation of the recording system.

METHODS

ACUTE EXPERIMENTS IN ANESTHETIZED DOGS

Six, adult, mongrel dogs of either sex were used in these experiments. The animals were fasted 24 hours before experimental use. Anesthesia was produced with pentobarbital sodium (30 mg/kg, IV) and a laparotomy was performed. A strain gauge (R.B. Products) was sutured (00 Ethicon suture) to the transverse axis of either the stomach (N=3), duodenum (N=2) or proximal large bowel (N=1) of the six dogs. The incision was closed with stainless steel wound clips and the electrical leads from the strain element attached through an electrical bridge to an oscillograph (Hewlett-Packard) for recording of circular muscle activity.

Primaquine diphosphate, dissolved in deionized water, was administered either through a stomach tube (PO) or intraluminally through a polyethylene catheter (PE 50) inserted into the lumen of the intestine, from a position rostral to the gauge, and advanced so that the tip of the catheter rested beneath the strain gauge. Epinephrine HCl and methacholine HCl were administered through a femoral vein cannula. In one dog a saturated ferric chloride solution, a powerful irritant, was administered PO.

Recording was started immediately after the completion of surgery and continued at 15 minute intervals for 6 to 8 hours post-primaquine administration.

EXPERIMENTS IN CONSCIOUS DOGS

Four, adult, mongrel dogs of either sex were used. The strain gauge was attached to the following levels of the gut:

Dog #7809 - on the stomach, 4 cm rostrally to the pylorus

Dog #7431 - on the small intestine, 300 cm caudally to the pylorus

Dog #8803 - on the small intestine, 40 cm caudally to the pylorus

Dog #8926 - on the terminal ileum, 2 cm rostrally to the ileocolic junction

The gut was exposed through a mid-line incision, the gauge attached transversely (00 Ethicon sutures) and the electrical leads were threaded through a skin tunnel to exit from a small incision located in the mid-line of the back at the anterior border of the scapulae. The dog was fitted with a leather harness and the electrical plug on the strain gauge leads was tied to the harness. During recording, the dogs were caged but otherwise unrestrained. In an attempt to prevent infection, the dogs received procaine penicillin at operation and every 5 days following surgery.

Dog 7809 was treated with 20 mg/kg primaquine diphosphate, PO, five days after surgery and was euthanized and autopsied 3 days later.

Dog 7431 received 3 separate doses of primaquine diphosphate, 10 mg/kg, PO, over a 6 week period the first dose being administered 16 days post-surgery.

Dog 8803 was treated, PO, with 10/kg primaquine diphosphate 16 days after surgery.

Due to technical difficulties, Dog 8926 was sacrificed before any drug administration.

Primaquine was always given after a 24 hour fast.

Records from dogs 7431 and 8803 were analyzed by calculating the percentage of time occupied by the 3 types of intestinal activity, basal, burst and intermediate, as described by Reinke (2).

RESULTS

ANESTHETIZED DOGS

The experiments in anesthetized dogs tended to confirm our previous observations that anesthesia is able to obtund intestinal drug reactions. Intravenous epinephrine, 1 μ g/kg, and methacholine, 1 μ g/kg, were completely ineffective in changing tone or contractions of the gut in 4 of the 6 dogs. Local intraluminal instillation of these drugs was not consistently effective either.

In the 2 dogs in which the injection of the standard pharmacological agents was effective, primaquine had no effect. In both of these 2 dogs the

strain gauge was sewn to the duodenum. In one of these dogs 50 mg/kg of primaquine diphosphate was administered orally and in the other animal two intraluminal instillations of 100 mg of primaquine diphosphate in 2 cc of deionized water were made followed by a serosal application of 500 mg of the primaquine salt.

CONSCIOUS DOGS

In dog 7809 the strain element was attached to the stomach. Five days were allowed for recovery from surgery prior to treatment with primaquine. Oral treatment with 20 mg/kg primaquine diphosphate, 440 mg/20 cc deionized water, followed by a 10 cc catheter flush had no immediate observable effect on gastric contractility or tone. Forty minutes after treatment the dog retched and vomited. Gastric contractions were recorded during vomiting. The dog again vomited 90 minutes after treatment. During the next three days, the dog ate very little. Activity types observed during this period were typical for a fasted dog. The dog was euthanized on the third post-treatment day.

Experiments in dog 7431 were carried out over a six week period. The animal received three 10 mg/kg doses of primaquine during this time. None of the treatments had an observable effect on intestinal tone or activity although recordings were taken for at least two to four hours post drug administration and at intervals until the animal was deemed ready for another dose.

Dog 8803 was treated with one oral dose of primaquine diphosphate, 10 mg/kg. The dog vomited two hours later. The strain gauge recorded intestinal movement associated with the vomiting. As in the other experiments, there was no observable effect of primaquine on tone or activity of the bowel.

Analysis of the records for the possible effect of primaquine on the percentage of time spent in the three primary activity types did not show any action of primaquine on these parameters.

At autopsy, gross inspection of the entire gastrointestinal tract was made. There was no evidence of infection in either the primary abdominal wound or the abdominal cavity of any of the chronic dogs. In all dogs fibrous tissue had completely encapsulated both the transducer and its electrical leads. There were numerous adhesions of the small bowel in dog 7431 perhaps indicating peritonitis at an earlier time.

Animal 8803 was autopsied 24 hours after primaquine treatment. No gross tissue damage was evident. In dogs 7809 and 7431, animals which were killed 3 and 9 days respectively after the final primaquine treatment, significant intestinal damage was observed.

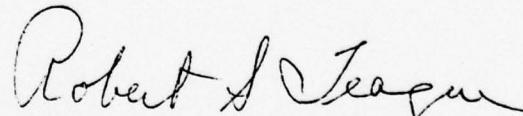
In dog 7809 the mucosal tissue of the stomach showed numerous patches of submucosal hemorrhage. The small intestines had numerous petechial hemorrhages on the serosal surface only. The damage became progressively less severe caudally. Only the proximal portion of the large bowel was damaged. Similar results were found in dog 7431. The damage was to the serosal surface of the gut and began at the terminal portion of the jejunum and progressed through the ileum and proximal large bowel.

DISCUSSION

Results from the experiments reported here are negative. It is not certain whether they should be interpreted to mean that oral doses of 10 mg/kg of primaquine diphosphate are without effect on gastrointestinal tone and activity or that the measurement technique is inadequate to detect changes in these parameters. An argument can be made against the latter assumption since changes in tone and circular muscle contractions were clearly recorded during vomiting. It was also possible for us to see activity patterns which have been described by other workers as being characteristic for the "fed" or "fasted" state in these animals. We take this to mean that the transducer system was recording faithfully. It was also possible, in experiments where it was tried, to record the effect of morphine on gastrointestinal tone.

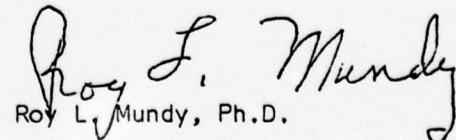
The experiments may be criticized on the basis that too low a dose was used. However, 10 mg/kg of primaquine diphosphate is a dose large enough to produce vomiting in most dogs and loss of appetite for a number of days post-administration in all.

Technical difficulties were frequently encountered in these experiments. The most common malfunction was caused by breakage of lead wires to the transducer. At least two experiments were terminated (although the experimental animal was in good condition) when lead wires parted deep within the animal. Also, there was difficulty in deciding how much of the gross pathology observed was due to drug administration. Although there were no overt signs of abdominal infection, "stress" caused by the indwelling strain gauge and its connector wires may have been involved in the serosal damage seen.



Robert S. Teague, M.D., Ph.D.

Professor & Chairman



Roy L. Mundy, Ph.D.

Professor

REFERENCES

1. Jacoby, H. I. et al. In Vivo Extraluminal Contractile Force Tranducer for Gastrointestinal Muscle, J. Appl. Physiol., 18:658, 1963
2. Reinke, D. A. et al. Patterns of Dog Gastrointestinal Contractile Activity Monitored in Vivo with Extraluminal Force Tranducers, Am. J. Dig. Dis., 12 (2): 113, 1967.



the University of Alabama in Birmingham/ UNIVERSITY STATION / BIRMINGHAM, ALABAMA 35294
the Medical Center /DEPARTMENT OF PHARMACOLOGY /January 26, 1977

ANTAGONISM OF PRIMAQUINE TO INTRAPERITONEAL ACETIC ACID
IN MOUSE WRITHING TEST

ROBERT S. TEAGUE

ROY L. MUNDY

Contract Number

DADA 17-67-C-7136

Interim Report Number 10

Fourth Quarter, 1976

ABSTRACT

Primaquine diphosphate was injected i.p. in mice to see if it produced pain like acetic acid or phenylquinone in a writhing test. It was thought that if the drug were an irritant this might shed light on its production of occasional GI symptoms in man on oral administration.

Primaquine injections of 10^{-4} or 10^{-3} M in doses of 0.02 ml/gm failed to produce writhing or behavior suggesting pain. An experiment was then devised to see if primaquine orally would be painful and thereby potentiate the writhing frequency produced by doses of acetic acid i.p.

In controls given saline orally followed by i.p. acetic acid, a dose-response relationship was observed among doses of 18.75, 37.5 and 75.0 mg/kg in 0.01 ml/gm body weight.

Primaquine orally in doses of 15 mg/kg (expressed as the base) had no effect on acetic acid-induced writhing as compared with controls, but at a dose of 30 mg/kg primaquine apparently displayed analgesic activity. Instead of potentiating the writhing response, this dose of primaquine attenuated by 51% the writhing frequency of mice treated with 37.5 mg/kg and by 40% those treated with 75 mg/kg of acetic acid.

The mechanism of this protective or antagonist action was not explained.

TO: Department of Pharmacology, Walter Reed Army Institute of Research,
Walter Reed Army Medical Center, Washington, D.C. 20012

SUBJECT: ANTAGONISM OF PRIMAQUINE TO INTRAPERITONEAL ACETIC ACID IN THE MOUSE
WRITHING TEST

INTRODUCTION

Orally administered primaquine in man may be accompanied by gastro-intestinal complaints including epigastric pain, nausea and diarrhea (1,2). It was hypothesized that if this pain was a result of a local irritant action on the GI tract it might cause writhing intraperitoneally in a mouse in a manner similar to that produced by intraperitoneal (i.p.) acetic acid and phenylquinone. Even if i.p. primaquine alone did not produce writhing, if given orally it might potentiate the writhing caused by acetic acid. Experiments were designed to test this hypothesis; the findings were contrary to expectation.

METHODS

Male, white, Swiss mice from a non-inbred colony (Southern Animal Farms, Prattville, Alabama) were used in all experiments. Animals were housed on hardwood bedding with food and water available ad libitum. Twenty-four hours prior to use, groups of mice were removed from home colony cages, placed in a wire bottom metabolism cage and fasted overnight. Water only was available during the fasting period.

Primaquine diphosphate was dissolved in de-ionized water in concentrations such that 0.02 ml/gm body weight delivered the desired dose of primaquine. Primaquine or in controls, an equal volume of 0.9% NaCl was administered by gavage. One hour later one of three doses of acetic acid, 18.75, 37.5 or 75.0 mg/kg, in 0.01 ml/gm was injected i.p. Glacial acetic acid was diluted freshly daily with de-ionized water to prepare the solutions. After placing the mice in individual shoe box cages, 5 minutes was allowed for the effect of the irritant to begin. This was followed by a 20 minute observation period during which the frequency of writhing was counted. A writhe is commonly considered to represent a characteristic sequence of behavior including arching of the back, pelvic rotation and extension of the hind limbs repeated at intervals (3). In these experiments, only writhes which included all three components and culminated in hind limb extension were counted.

Data were analyzed by the analysis of variance and by Student's t test.

RESULTS

Experience in this laboratory had indicated that primaquine alone would not cause writhing in mice. This prediction was confirmed in preliminary writhing experiments with primaquine. Primaquine concentrations of 1×10^{-4} or 1×10^{-3} M injected i.p. failed to produce typical writhing in mice and their behavior was no different than mice injected with an equal volume of 0.9% NaCl.

A dose-response relationship of writhing frequency and acetic acid concentration was observed to doses of 18.75, 37.5 and 75 mg/kg i.p. (Table 1). At doses above 75 mg/kg of acetic acid no further increase in frequency of writhing was observed. All mice tested survived acetic acid treatment with doses as high as 300 mg/kg.

The frequency of acetic acid-induced writhing varied considerably from day to day. In control animals receiving a dose of 37.5 mg/kg of acetic acid (Table 1) it should be noted that in one group of animals writhing frequency was only 58% of the other group. As noted by Taber (3) this is a common problem with this test but not one which severely limits its use. It is necessary however to design experiments so that both control and treated animals are injected and observed concurrently and that experiments testing any one dose of irritant be completed in as short a time span as possible.

Primaquine in doses of 15 mg/kg (expressed as the base) had no effect on acetic acid-induced writhing (Table 1). However, at a dose level of 30 mg/kg primaquine displayed apparently analgesic properties. This dose attenuated by 51% the number of writhes exhibited by mice treated with 37.5 mg/kg of acetic acid and by 40% those treated with 75 mg/kg (Table 1).

DISCUSSION

A one hour oral pretreatment of mice with primaquine diphosphate diminished the frequency of writhing after intraperitoneally administered acetic acid. The analgesic action of primaquine in these experiments was somewhat dose-related, in that a dose of 15 mg/kg of primaquine was ineffective whereas a larger dose of 30 mg/kg significantly attenuated writhing frequency.

To label primaquine an analgesic may however be inappropriate. The mouse writhing test though extremely sensitive is highly non-specific. Many compounds which are ineffective as analgesics in clinical medicine will inhibit writhing in the mouse. A partial listing of such compounds includes lidocaine, pentylenetetrazol, ephedrine, tripeleannamine, physostigmine, pilocarpine, phenoxybenzamine (4), amphetamine, chlorpromazine (5), tranylcypromine and homochlorcyclizine (6). This laboratory has previously reported that primaquine possesses anticholinergic properties (7) but Hendershot and Forsaith (4) reported that atropine affords little or no protection against phenylquinone-induced writhing.

On the other hand, quinine is known to have local anesthetic and antifibrillatory properties and Hendershot and Forsaith (4) found that lidocaine, injected subcutaneously, was moderately effective in protecting against phenylquinone-induced writhing. The mechanism of this protective action is not understood but it is possible that primaquine inhibits writhing in the same manner as does lidocaine.

Thus, although very sensitive the mouse writhing test lacks specificity as a test for analgesics. A large number of compounds with a variety of pharmacological actions are detected as "false positives." Because of the broad spectrum of physiological mechanisms exhibited by these "false positives" it is impossible to deduce from these experiments the mechanism of action of primaquine's apparent analgesic action in this test.

Ray L. Munday

Robert S. League

REFERENCES

1. Alving, A.S., Pullman, T.N., Craige, B., Jones, R., Whorton, C.M., Eichelberger, L., The clinical trial of eighteen analogues of pamaquin (Plasmochin) in vivax Malaria (Chesson Strain), J. Clin. Invest., 27: 34-45, 1948.
2. Edgecomb, J.H., Arnold, J., Yount, E.H., Alving, A.S., Eichelberger, L., Primaquine, SN 13272, A new curative agent in vivax Malaria: A preliminary report, J. Natl. Malaria Soc., 9:285-292, 1950.
3. Taber, I., Predictive value of analgesic assays in mice and rats. In: Narcotic Analgesics, edited by M.C. Braude, et al. Advances in Biochemical Psycho-pharmacology, Vol. 8, Raven Press, New York, 1974.
4. Hendershot, L.C. and J. Forsaith, Antagonism of the frequency of phenyl-quinone-induced writhing in the mouse by weak analgesics and nonanalgesics, J. Pharm. Expt. Therap. 125:237-240, 1965.
5. Pearl, J., H. Stander and D.B. McKean, Effects of analgesics and other drugs on mice in phenylquinone and rotarod test, J. Pharm. Expt. Therap. 167: 9-13, 1969.
6. Brattain, R.T., D.N. Lehrer and P.S.J. Spencer, Phenylquinone writhing test: Interpretation of data, Nature, 200:895-896, 1963.
7. Teague, R.S. and R.L. Mundy, Effect of primaquine on the sensitivity of guinea pig ileum to acetylcholine and histamine, Interim Report No. 1, A Study of Adverse Gastrointestinal Effects of 8-Aminoquinolines. Annual Progress Report, 1973-1975.

TABLE 1

The effect of oral primaquine treatment one hour before intraperitoneal acetic acid-induced writhing in the mouse. Numbers in each cell represent the mean number of writhes, \pm S.E.M., per 20 minute observation period. Numbers in parentheses indicate the number of animals used in each experiment.

EXPERIMENT	TREATMENT	ACETIC ACID DOSE (mg/kg)		
		18.75	37.5	75.0
WRITHING FREQUENCY				
I	NaCl		16.3 \pm 6.3 (7)	37.4 \pm 9.9 (8)
	Primaquine 15 mg/kg		13.4 \pm 3.8 (7)	27.1 \pm 5.0 (7)
II	NaCl	1.1 \pm 1.1 (8)	28.2 \pm 5.5 (13)	35.6 \pm 3.2 (15)
	Primaquine 30 mg/kg	2.0 \pm 1.9 (8)	*13.7 \pm 3.3 (15)	*21.3 \pm 3.6 (15)

* $P < 0.05$ as compared with its respective control mean



the University of Alabama in Birmingham/UNIVERSITY STATION / BIRMINGHAM, ALABAMA 35294
the Medical Center/DEPARTMENT OF PHARMACOLOGY /February 9, 1978

ANTIMUSCARINIC ACTIVITY OF THE 8-AMINOQUINOLINE ANTIMALARIAL DRUG,
PRIMAQUINE, ON THE GALL BLADDER OF THE GUINEA PIG

ROBERT S. TEAGUE

ROY L. MUNDY

Contract Number

DADA 17-67-C-7136

Interim Report No. 11

ABSTRACT

The thesis that primaquine might cause epigastric distress by initiating spasm of the gall bladder has been tested by measuring the change in pressure in the isolated guinea pig gall bladder in vitro following the addition of primaquine to the bathing solution. Atropine, when used as a positive control, is a potent competitive inhibitor of cholinergic stimulation of the preparation. Primaquine has a weak competitive antimuscarinic action.

This pharmacological action of primaquine cannot be used to explain the production of epigastric discomfort following the oral ingestion of primaquine by man and the thesis is rejected. If an antimuscarinic action accounted for epigastric distress, atropine should be a highly active distress causing agent.

TO: Department of Pharmacology, Walter Reed Army Institute of Research,
Walter Reed Army Medical Center, Washington, D.C. 20012

SUBJECT: ANTIMUSCARINIC ACTIVITY OF THE 8-AMINOQUINOLINE ANTIMALARIAL DRUG,
PRIMAQUINE BY THE GALL BLADDER OF THE GUINEA PIG

INTRODUCTION

One of the primary gastrointestinal symptoms following oral administration of primaquine in man is epigastric discomfort (1). Spasm of the gall bladder might account for this effect. An in vitro system was developed, the measurement of change in pressure in the isolated guinea pig gall bladder, to test the hypothesis.

METHODS

Twelve, adult, male, Hartley guinea pigs, weighing from 350-450 grams, were sacrificed by cervical dislocation. The gall bladder was quickly removed and placed in room temperature ($23 \pm 2^\circ C$) Tyrode's solution (2). Under low magnification the cystic duct was cut approximately two mm distal to the junction of the duct and the neck of the gall bladder. The duct was dilated with fine forceps and a half-inch long blunt, beveled, steel cannulae (18 gauge hypodermic needle) inserted and tied in place. After removal of air bubbles by holding the needle upright and manual manipulation, the bladder was immersed in a chamber containing 20 ml of Tyrode's solution and the cannula attached to a 1280A Sanborn (Hewlett-Packard) pressure transducer (Serial No. DK) with a calibration factor of 15 MV/V.F.S. via a 30 cm length of Tyrode-filled tygon tubing (Intramedic^R (Clay-Adams) PE20, ID 0.015 inches, OD 0.045 inches, which had been tested for animal experimentation). The transducer was energized and the signal amplified by a Sanborn 350-1100C (Hewlett-Packard) carrier preamplifier which was connected to a driver amplifier and power supply model 150-200B/400 (Hewlett-Packard) which supplied the signal to a galvanometer in the Hewlett-Packard, Series 7700, heat-writing recorder. The chamber was maintained at $32^\circ C$ in a ten gallon constant temperature bath (aquarium, Metaframe Corporation, 41 Slater Drive, Elmwood Park, NJ 07407) (stirred and heated by a Thermomix^R 1420, B. Braun, Melsunger AG, Model IP 21, thermostatically controlled heating and stirring apparatus), and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The transducer and electrical recording system was calibrated by applying known pressures from a water manometer to the transducer.

A stopcock, 5 cm below the level of the bladder, was opened so that slight negative pressure emptied the gall bladder. The stopcock was closed and each bladder filled to a 100 μl volume. This volume produced an intraluminal pressure of approximately 100 mm of water (100 ± 15 mm). Drug-induced changes in pressure were measured as increases above this initial pressure.

Tissues were allowed to equilibrate in the chamber for thirty minutes during which time they were washed by overflowing 60 ml of fresh Tyrode's solution through the chamber two times. The experiments consisted of determining the maximal response of the guinea pig gall bladder to acetylcholine ($10^{-6} - 10^{-2}$ M) added cummulatively in the absence of inhibitor and in the same tissue after the

addition of primaquine (either 10^{-4} M or 3.3×10^{-4} M) or atropine (10^{-8} or 3.3×10^{-8} M). The agonist was added at the lowest concentration used in the experiment and the tissue was allowed to equilibrate to a new pressure level and the next dose of agonist was then added and a new equilibrium of pressure attained. This procedure was continued until the pressure no longer increased following drug addition or the pressure decreased following an incremental addition. The equilibrium point between concentrations was generally reached in approximately 2 minutes. The tissue was then washed free of agonist by overflowing two 60 milliliter quantities of Tyrode's solution through the muscle chamber. When the preparation had reached its original pressure level following washing, the antagonist was administered in the stated concentration, 10 minute contact time elapsed, and the agonist doses were again administered as described above. Naturally, since an inhibitor was present, it was necessary to use larger concentrations of the agonist to attain a maximal contraction of the tissue (Figure 1). There was no indication that the bladder preparation deteriorated during the testing procedure. Preliminary experiments involving multiple determinations of agonist dose-response characteristics, showed that the preparation did not lose sensitivity to acetylcholine during the time period used in these experiments.

RESULTS

Figure 1 illustrates the results of antagonism of the agonist, acetylcholine, by 10^{-4} M primaquine. The dose-response curve obtained in the presence of primaquine is shifted to the right indicating an antagonistic action of primaquine on acetylcholine-induced contraction of the gall bladder. The two dose-response curves appeared to be parallel and there was no diminution of maximal response. Similar experimental results were obtained with a higher (3.3×10^{-4} M) concentration of primaquine diphosphate and with two concentrations of atropine sulfate (10^{-8} and 3.3×10^{-8} M).

The antagonism produced by primaquine and atropine appears to be competitive as evidenced by the parallel shift in the dose-response curves and failure of either compound to reduce the response maximum. The idea of competitive inhibition was strengthened through analysis of the data by double reciprocal plots (Figures 2 and 3). These plots were linear in all cases and exhibited similar maxima in all studies. Non-competitive antagonism would have yielded plots with divergent maxima.

In order to compare potencies of the two antagonists, pA_2 values were calculated by the method of Schild (3). The pA_2 of atropine (10 min. contact) was 8.57 while that for primaquine (10 min. contact) was much less potent, 4.57 (Figure 4).

DISCUSSION

Modern concepts in pharmacodynamics allow the classification of the mechanism of action of antagonists into at least two broad categories of competitive and non-competitive antagonists. Criteria for this classification system have been outlined by Schild (4). In the work reported here several of the criteria required to classify primaquine diphosphate as a competitive antagonist

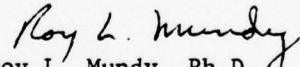
of acetylcholine on the guinea pig gall bladder have been met. First, the plot in Figure 1 shows that the contractile response of the gall bladder to increasing concentrations of acetylcholine is dose-related and that the addition of 10^{-4} M concentrations of primaquine to the organ bath decreases the contractile responses to the same concentrations of the agonist. The fact that the dose-response curve is shifted to the right in a parallel manner and the maximum response does not appear to be diminished gives us the first suggestion that we are dealing with a competitive antagonist. Second, the test of Lineweaver and Burk (5) allows us to determine that the maxima of the contractile responses are not depressed in our experiments and third, tests based on a comparison of equi-active doses (pA_2) revealed that both antagonists (primaquine and atropine) gave a straight line relationship in plots of dose-ratios - 1 versus the negative logarithm of the concentration of the antagonist with parallel slopes. This test also allows a quantitative statement of the potency of the antagonist.

The pA_2 value for atropine is in good agreement with the pA_2 for atropine as obtained by Schild in 1947 (3). He found the 2-minute pA_2 for atropine to be 8.37 and the 14-minute (contact time) pA_2 to be 8.77. In our experiments a ten-minute contact time with atropine produced a pA_2 value of 8.57. Primaquine diphosphate is thus a much less potent muscarinic receptor inhibitor than is atropine.

Although this paper demonstrates an antimuscarinic effect of primaquine in the guinea pig gall bladder it does not explain the epigastric distress caused by the agent. Since Ach is an excitatory transmitter in the gall bladder, inhibition of muscarinic receptors with primaquine would tend to relax rather than cause spasm in the gall bladder musculature.



Robert S. Teague, M.D.
Principal Investigator


Roy L. Mundy
Roy L. Mundy, Ph.D.
Co-Investigator

REFERENCES

1. Edgcomb, John H., Arnold, John, Yount, Ernest, H., Jr., Alving, Alf S. and Eichelberger, Lillian. Primaquine, SN 13273, A New Curative Agent In Vivax Malaria: A Preliminary Report, J. Nat. Malaria Soc. 9:285-292, 1950.
2. Pharmacological Experiments on Isolated Preparations, By the staff of the Department of Pharmacology, University of Edinburgh, E. and S. Livingstone, Ltd., Edinburgh and London, 1968, pp. 121.
3. Schild, H.O., pA, A New Scale for the Measurement of Drug Antagonism, Brit. J. Pharmacol. 2:189-206, 1947.
4. Schild, H.O., Drug Antagonism and pAx. Pharmacol. Reviews, 9:242-246, 1957.
5. Lineweaver, H. and Burk, D., The Determination of Enzyme Dissociation Constants, J. Am. Chem. Soc. 56:658-666, 1934.

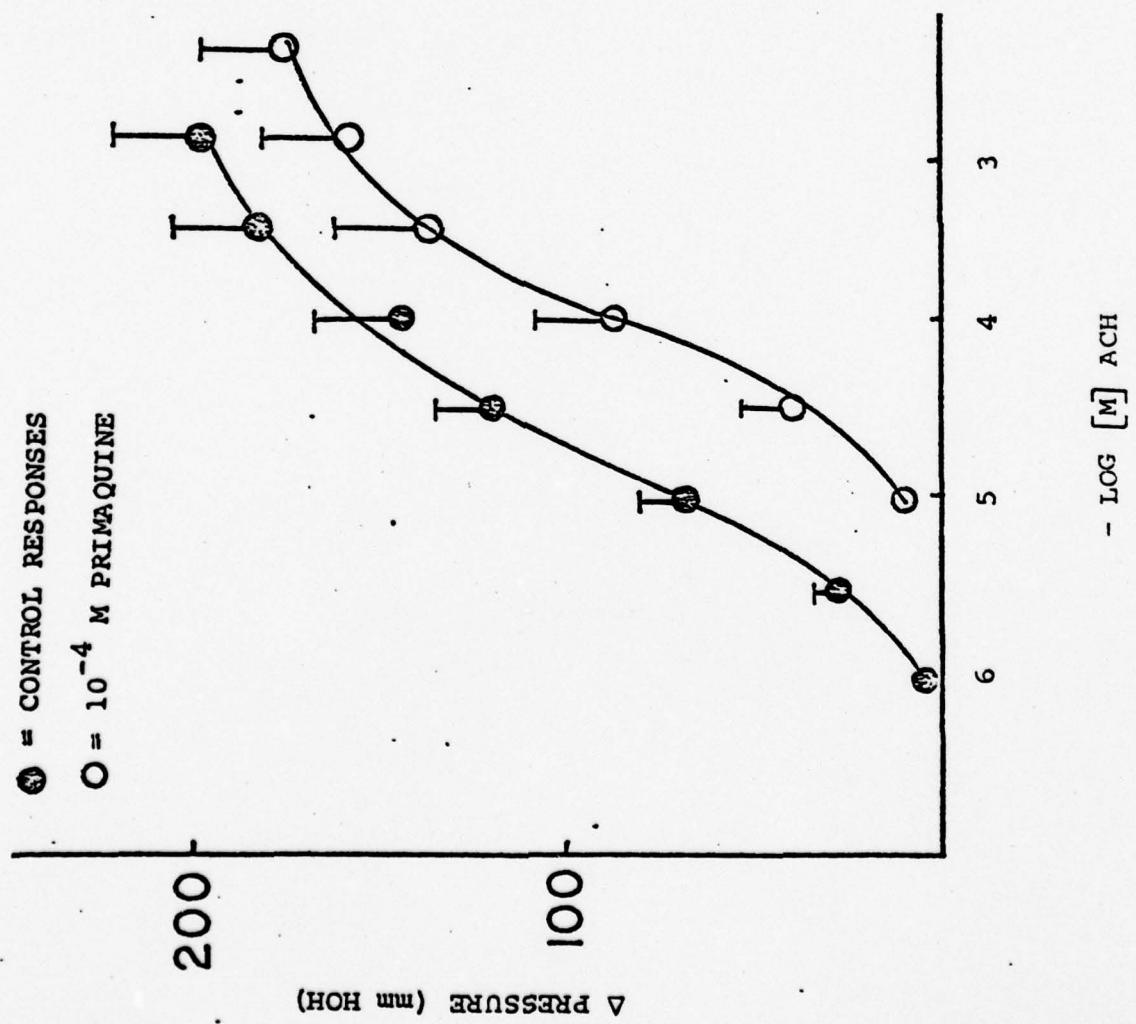


Figure 1. Antagonism by primaquine of the response of the guinea pig gall bladder to acetylcholine. Each point represents the mean response (+ 1 S.E.M.) of three gall bladders.

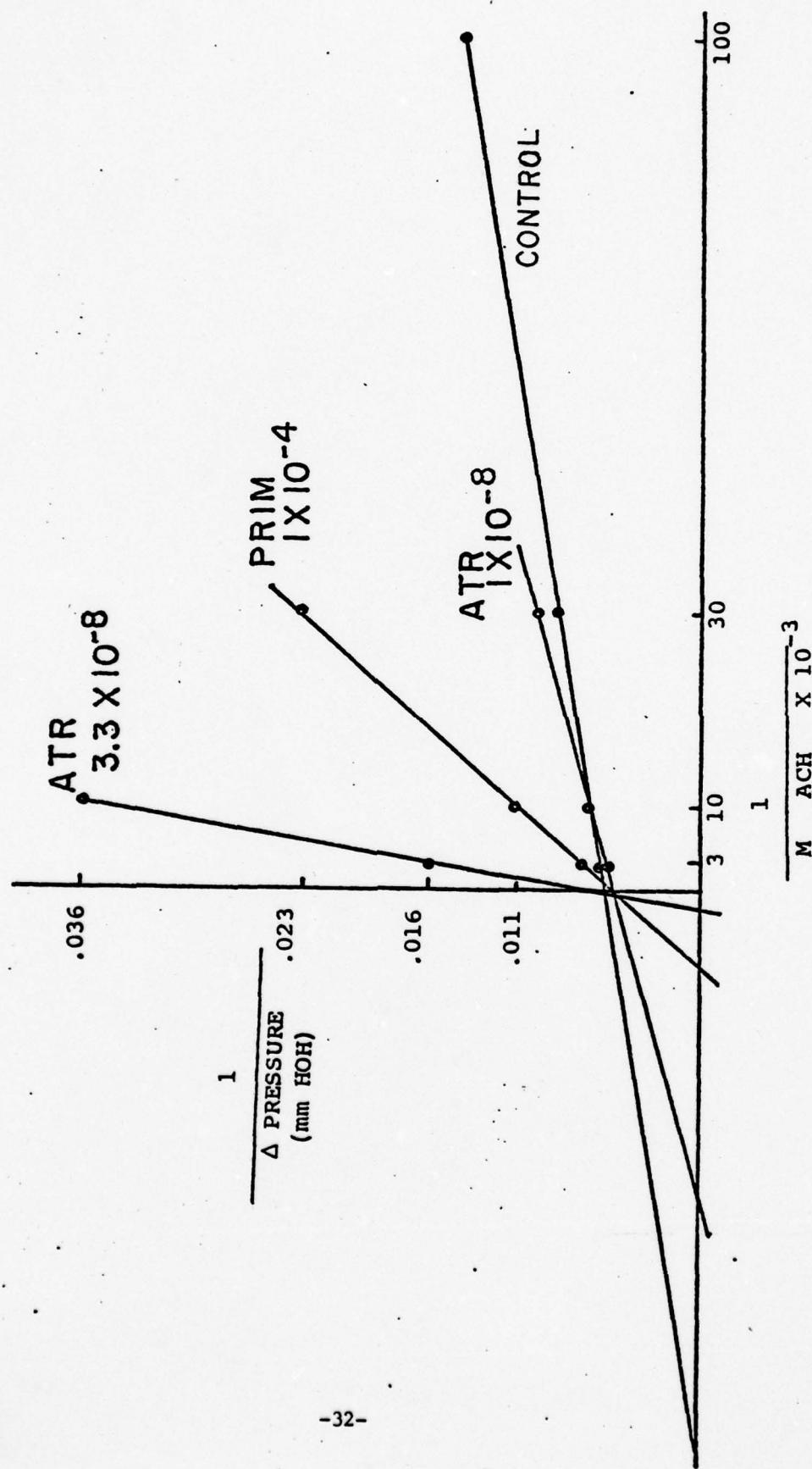


Figure 2. Double reciprocal plot of the antagonism by primaquine (PRIM) and atropine (ATR) of the response of the guinea pig gall bladder to acetylcholine. Each point represents the mean of three gall bladders. Control=responses to acetylcholine alone.

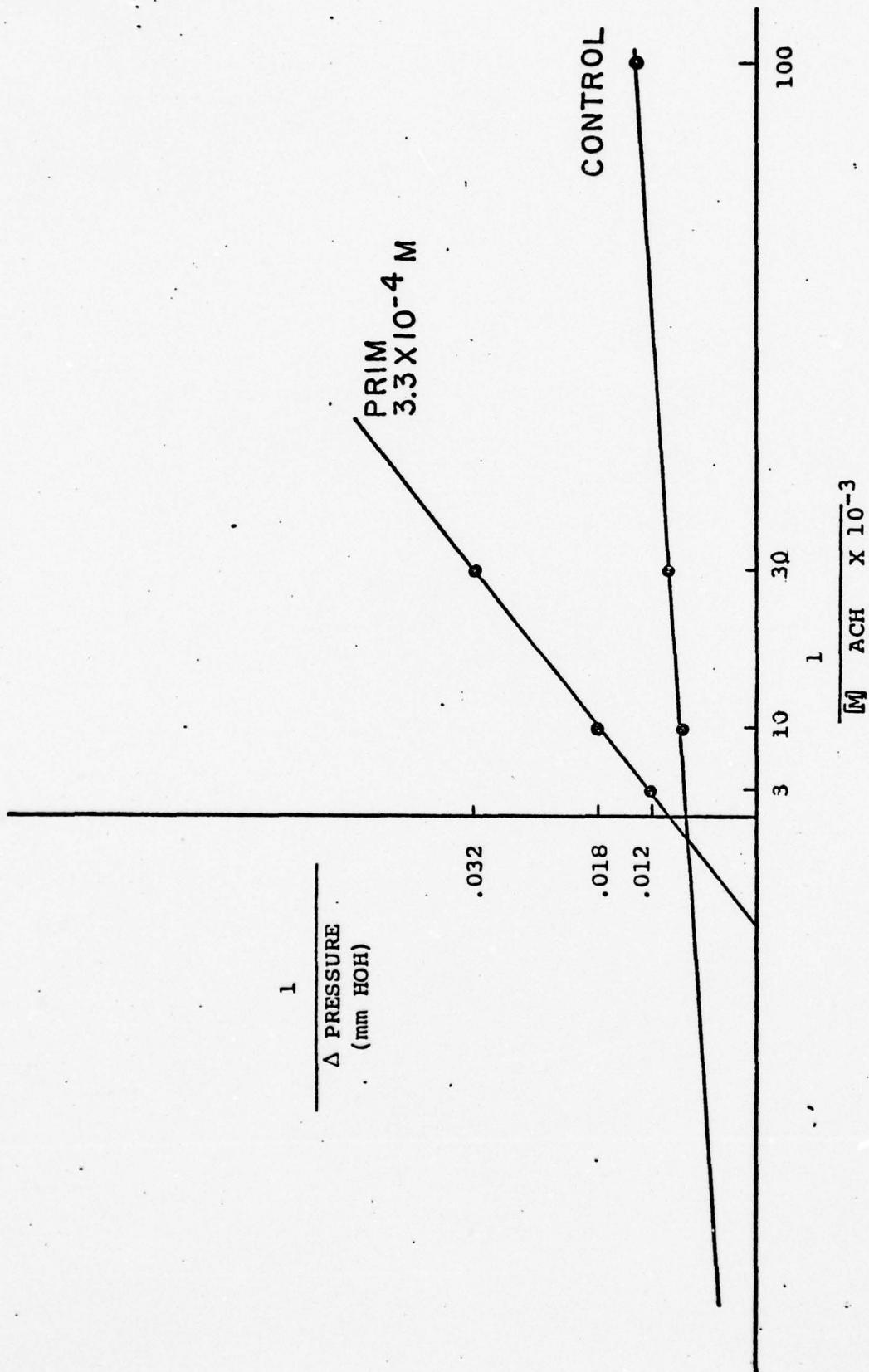


Figure 3. Double reciprocal plot of the antagonism by primugine (PRIM) of the response of the guinea pig gall bladder to acetylcholine. Each point represents the mean of three gall bladders. Control=responses to acetylcholine alone.

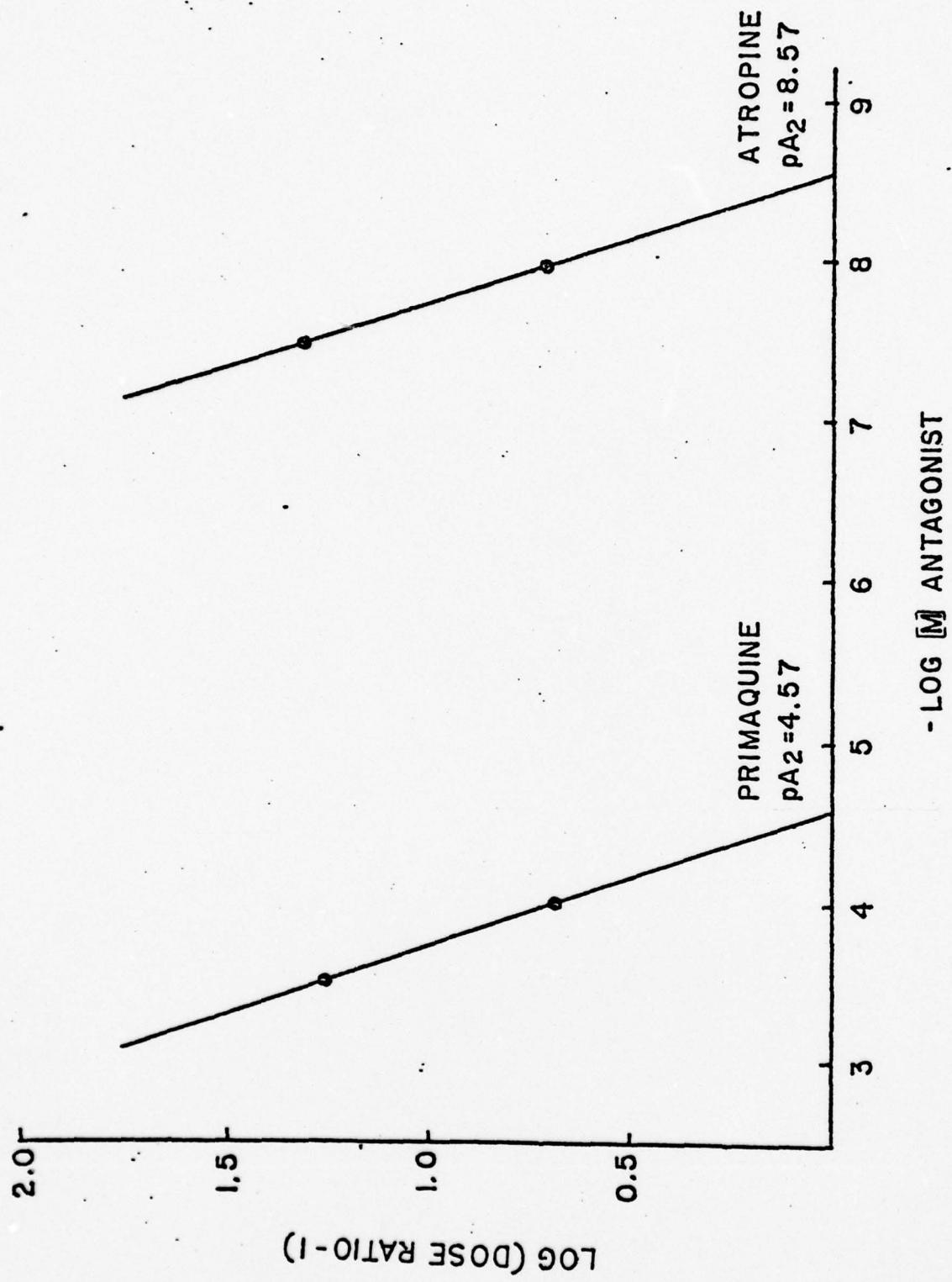


Figure 4. Plot of the \log (dose ratio - 1) against the negative \log of the concentration of antagonist. The PA_2 was graphically determined. The dose ratio is the ratio of concentration of acetylcholine required to produce a given response in the presence and absence of an antagonist.



the University of Alabama in Birmingham/ UNIVERSITY STATION / BIRMINGHAM, ALABAMA 35294
the Medical Center/ DEPARTMENT OF PHARMACOLOGY

1 March 1978

POTENTIATION OF THE EFFECTS OF ACETYLCHOLINE AND OXOTREMORINE ON THE
GALL BLADDER OF THE GUINEA PIG BY CHOLECYSTOKININ AND THE
INTERACTION OF PRIMAQUINE WITH CHOLECYSTOKININ

ROBERT S. TEAGUE

ROY L. MUNDY

Contract Number

DADA 17-67-C-7136

Interim Report No. 12

ABSTRACT

The interaction of primaquine and the C-terminal octapeptide of cholecystokinin was studied in the isolated guinea pig gall bladder. Primaquine was shown to be an antagonist of the peptide. The paper also reports a potentiating action of cholecystokinin with both acetylcholine and oxotremorine in the preparation.

TO: Department of Pharmacology, Walter Reed Army Institute of Research,
Walter Reed Army Medical Center, Washington, D.C. 20012

SUBJECT: POTENTIATION OF THE EFFECTS OF ACETYLCHOLINE ON THE GALL BLADDER
OF THE GUINEA PIG BY CHOLECYSTOKININ AND THE INTERACTION OF
PRIMAQUINE WITH CHOLECYSTOKININ

INTRODUCTION

The rationale for this work is that the production of epigastric distress following the oral ingestion of primaquine diphosphate tablets by U.S. service personnel may be caused by disruption of the normal motor function of the gall bladder. A recent report from this laboratory has shown that primaquine is a weak antimuscarinic agent (1). This attribute would not account for the adverse mechanism of action of the primaquine because other potent antimuscarinic agents, e.g. atropine, do not produce epigastric distress. However, it is well known that cholinergic influences are not the only factors in gall bladder motor function(2).

Cholecystokinin, a duodenal peptide, is released by a variety of stimuli and travels via the blood stream to the gall bladder where it causes a vigorous contraction of the muscular wall of the organ and a concomitant relaxation of the sphincter muscle leading to gall bladder evacuation. Recently the C-terminal octapeptide of cholecystokinin (octa-CCK) has been made available for use in medicine as a gall bladder evacuant. The synthetic compound acts physiologically, generally, as the native peptide (3).

It was of interest to us to determine the effect of primaquine diphosphate on the action of octa-CCK on the isolated gall bladder of the guinea pig. If primaquine acted synergistically with octa-CCK this action could lead to gall bladder spasm and result in discomfort. Of course, it is obvious that disruption of the normal motor control by an antimuscarinic and anti-octa-CCK action could lead to a "limp" gall bladder which, if distended, could cause distress.

METHODS

Forty-eight, adult, male, Hartley guinea pigs, weighing from 350-450 grams, were sacrificed by cervical dislocation. The gall bladder was quickly removed and placed in room temperature ($23 \pm 2^\circ$ C) Tyrode's solution (4). Under low magnification the cystic duct was cut approximately two mm distal to the junction of the duct and the neck of the gall bladder. The duct was dilated with fine forceps and a half-inch long blunt, beveled, steel cannulae (18 gauge hypodermic needle) inserted and tied in place. After removal of air bubbles by holding the needle upright and manual manipulation the bladder was immersed in a chamber containing 20 ml of Tyrode's solution and the cannula attached to a 1280A Sanborn (Hewlett-Packard) pressure transducer (Serial No. DK) with a calibration factor of 15 MV/V.F.S. via a 30 cm length of Tyrode-filled tygon tubing (Intramedic^R (Clay-Adams) PE 20, ID 0.015 inches, OD 0.045 inches, which had been tested for animal experimentation). The transducer

was energized and the signal amplified by a Sanborn 350-1100C (Hewlett-Packard) carrier preamplifier which was connected to a driver amplifier and power supply model 150-200B/400 (Hewlett-Packard) which supplied the signal to a galvanometer in the Hewlett-Packard, Series 7700, heat-writing recorder. The chamber was maintained at 32° C in a ten gallon constant temperature bath (aquarium, Metaframe Corporation, 41 Slater Drive, Elmwood Park, NJ 07407) (stirred and heated by Thermomix^R 1420, B. Braun, Melsurger AG, Model IP 21, thermostatically controlled heating and stirring apparatus), and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The transducer and electrical recording system was calibrated by applying known pressures from a water manometer to the transducer.

A stopcock, 5 cm below the level of the bladder, was opened so that slight negative pressure emptied the gall bladder. The stopcock was closed and each bladder filled to a 100 μ l volume. This volume produced an intraluminal pressure of approximately 100 mm of water (100 ± 15 mm). Drug-induced changes in pressure were measured as increases above this initial pressure.

Tissues were allowed to equilibrate in the chamber for thirty minutes during which time they were washed by overflowing 60 ml of fresh Tyrode's solution through the chamber two times. The initial experiments consisted of determining the maximal response of the guinea pig gall bladder to cumulative additions of acetylcholine (10^{-6} - 10^{-2} M), oxotremorine (10^{-4} - 10^{-7} M) and octa-CCK (10^{-7} - 3.3×10^{-9} M). The effect of octa-CCK (10^{-8} - 3.3×10^{-8} and 10^{-7} M) on the sensitivity of the gall bladder to acetylcholine (10^{-5} , 3.3×10^{-5} - 10^{-4} M) was studied by constructing a dose-response curve with the above stated doses of acetylcholine, washing the agonist from the bath and repeating the dose-response curve after the addition of the stated doses of octa-CCK. The peptide was allowed to remain in contact with the gall bladder for 10 minutes before the cumulative additions of the agonist were made. In all cases, the gall bladder was allowed to reach a pressure equilibrium before the next addition of agonist. Generally the pressure reached an equilibrium in approximately 3 minutes. The effect of octa-CCK (3.3×10^{-8} - 10^{-8} M) on the sensitivity of the preparation to oxotremorine (3.3×10^{-7} , 10^{-6} , 3.3×10^{-6} M) was studied using an identical protocol. Experiments on the interaction of octa-CCK and primaquine diphosphate were carried out by making cumulative additions (10^{-9} - 3.3×10^{-8} M) of octa-CCK to the gall bladder and washing out the agonist. When the agonist was washed out, the pressure in the gall bladder returned to its control level (100 ± 15 mm of water). Primaquine diphosphate was then added to the bath (10^{-4} M), 10 minutes were allowed to elapse and the doses of the agonist were again added cumulatively. This last dose series included a concentration of 10^{-7} M octa-CCK.

RESULTS

Figure 1 shows the relative potencies of acetylcholine, oxotremorine (oxo), a pure muscarinic agonist, and octa-CCK on the gall bladder of the guinea pig. Acetylcholine is a weak agonist in this preparation and has maximal activity at concentrations above 10^{-3} M. Oxotremorine was considerably more potent and had maximal activity at 3.3×10^{-5} M. The synthetic peptide was extremely potent and produced a detectable response at 3.3×10^{-9} M. Maximal responses were not attainable with the peptide because the concentrations available with the commercial pharmaceutical material were too low to allow preparation of higher concentrations of octa-CCK in the muscle bath.

Figure 2, again, shows the low potency of acetylcholine in the isolated guinea pig gall bladder. The figure also shows that gall bladders exposed to low concentrations of octa-CCK (10^{-8} - 10^{-7}) for a 10 minute period with subsequent washing, became more sensitive to acetylcholine. This result was obtained with no effect of the octa-CCK on resting pressure (tension) of the gall bladder.

Figure 3 shows that oxo is also potentiated by pretreatment with octa-CCK. The contraction produced by 10^{-6} M oxo is tripled by a 10 minute pretreatment of the gall bladder, followed by washout, with 10^{-7} M octa-CCK.

The results of experiments with octa-CCK and primaquine diphosphate are tabulated in Table 1. A dose-response curve for the octapeptide was determined by adding to the muscle bath concentrations ranging from 10^{-9} to 3.3×10^{-8} M. The peptide was subsequently washed out, and primaquine diphosphate 10^{-4} M was added (10 minute contact time) and the dose-response curve reconstructed by adding the original doses of octa-CCK. The second dose-response curve was shifted to the right in a parallel fashion. Double reciprocal plots of these data (not illustrated) indicated that the maximal response to the peptide was not diminished (4).

The octapeptide of cholecystokinin (Sincalide^R) was generously provided by E. R. Squibb and Sons, Princeton, New Jersey.

DISCUSSION

Acetylcholine is a much less potent agonist than octa-CCK in the isolated guinea pig gall bladder preparation. This finding agrees with information from whole animal experiments which show that stimulation of the vagus nerve causes only a weak contraction of the gall bladder. Pretreatment of the gall bladder with the humoral agent octa-CCK potentiated the action of the neurotransmitter, acetylcholine, in our preparation. It would be attractive to speculate that this interaction of a humoral agent with neurally released transmitter might provide a modulated control system for gall bladder evacuation.

Oxotremorine, a direct muscarinic receptor agonist, is not hydrolyzed by acetylcholinesterase. Its action was also potentiated by octa-CCK. Therefore we are provided with indirect evidence that the potentiation of the action of acetylcholine by octa-CCK is not via the inhibition of acetylcholinesterase by the peptide.

Preliminary experiments with primaquine indicate that it is a competitive antagonist of octa-CCK. It appears to shift the concentration-response curve to the right and not to diminish the maximum response obtainable.

Inhibition of the potency of both acetylcholine and cholecystokinin by primaquine might compromise normal gall bladder motor function. Such a "relative paralysis" might limit the ability of the structure to evacuate its contents and lead to distension of the gall bladder with activation of stretch receptors for pain.

Robert S Teague

Robert S. Teague, M.D.
Principal Investigator

Roy L. Mundy

Roy L. Mundy, Ph.D.
Co-Investigator

REFERENCES

1. Teague, R. S., and R. L. Mundy, Antimuscarinic activity of the 8-aminoquinoline antimalarial drug, primaquine, on the gall bladder of the guinea pig, Interim Report No. 11, Walter Reed Army Institute of Research, 1978.
2. Pallin, B. and S. Skoglund, Neural and humoral control of the gall bladder-emptying mechanism in the rat. *Acta Physiol. Scand.* 60:358-362, 1974.
3. Rubin, B., S.L. Engel, A.M. Drungis, M. Dzelzkalns, E.O. Grigas, M. H. Waugh and E. Yiacas. Cholecystokinin-like activities in guinea pigs and in dogs of the C-terminal octapeptide (SQ 19,844) of cholecystokinin. *J. Pharm. Sci.* 58:955-959, 1969.
4. Staff of the Department of Pharmacology, University of Edinburgh: Pharmacological Experiments on Isolated Preparations. E. & S. Livingstone, Ltds., Edinburgh and London, 1968, p. 121.
5. Lineweaver, H. and D. Burk, The determination of enzyme dissociation constants. *J. Amer. Chem. Soc.* 56:658-666, 1934.

TABLE 1

The response of the guinea pig gall bladder to octa-CCK in the presence and absence of 10^{-4} M primaquine.

Octa-CCK	ABSENCE	PRESENCE OF 10^{-4} M PRIMAQUINE
$10^{-9}*$	7.7 ± 1.5	1.3 ± 0.7
3.3×10^{-9}	28.2 ± 4.4	4.7 ± 2.7
10^{-8}	73.0 ± 15.0	28.2 ± 11.9
3.3×10^{-8}	83.3 ± 25.7	68.8 ± 20.9
$10^{-7}+$		112.7 ± 25.8

* molar concentration of Octa-CCK.

mean (\pm S.E.M.) of the responses to octa-CCK of three gall bladders.

+ highest dose of octa-CCK used in presence of inhibitor and not in control trials-
see Methods.

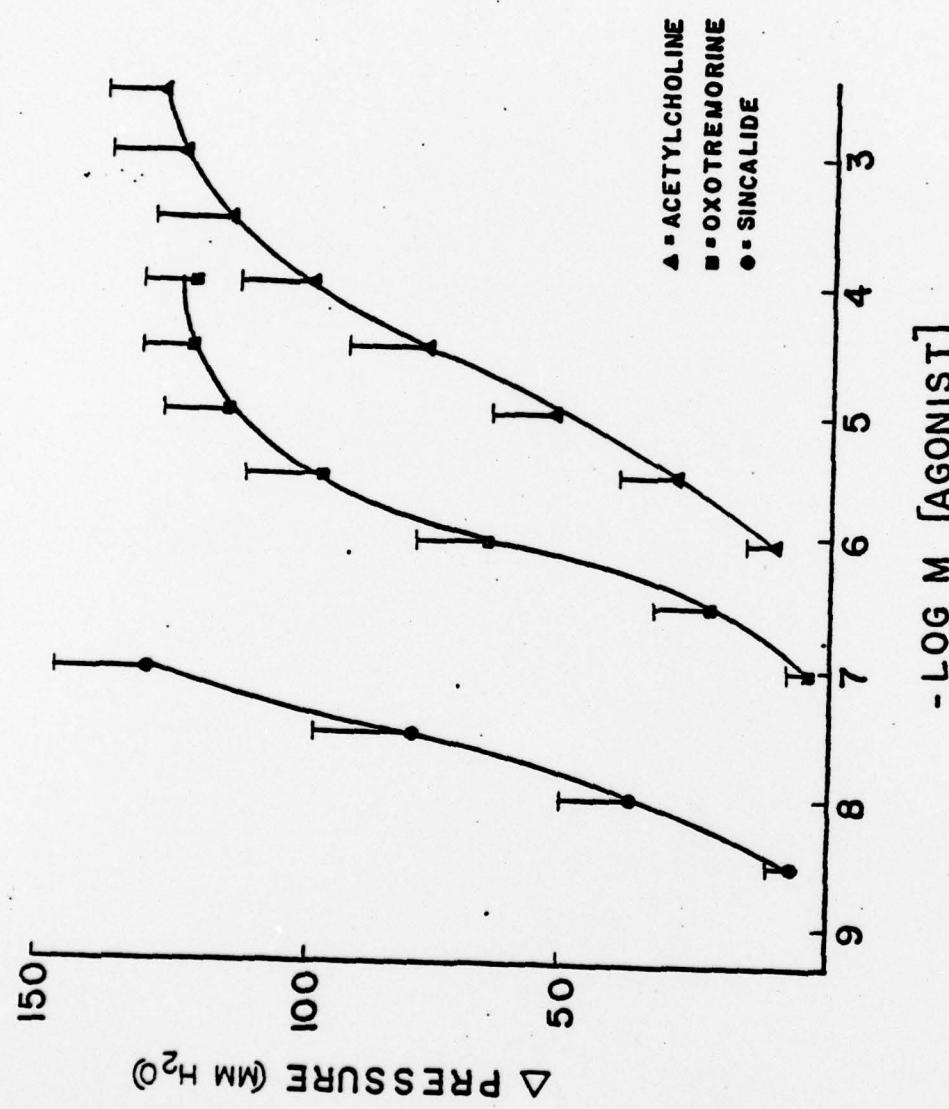


Figure 1. Control dose response curves for 3 agonists in the isolated guinea pig gall bladder preparation. Each point represents the mean (+ 1 S.E.M.) of three gall bladders. Pressure was measured in mm of water.

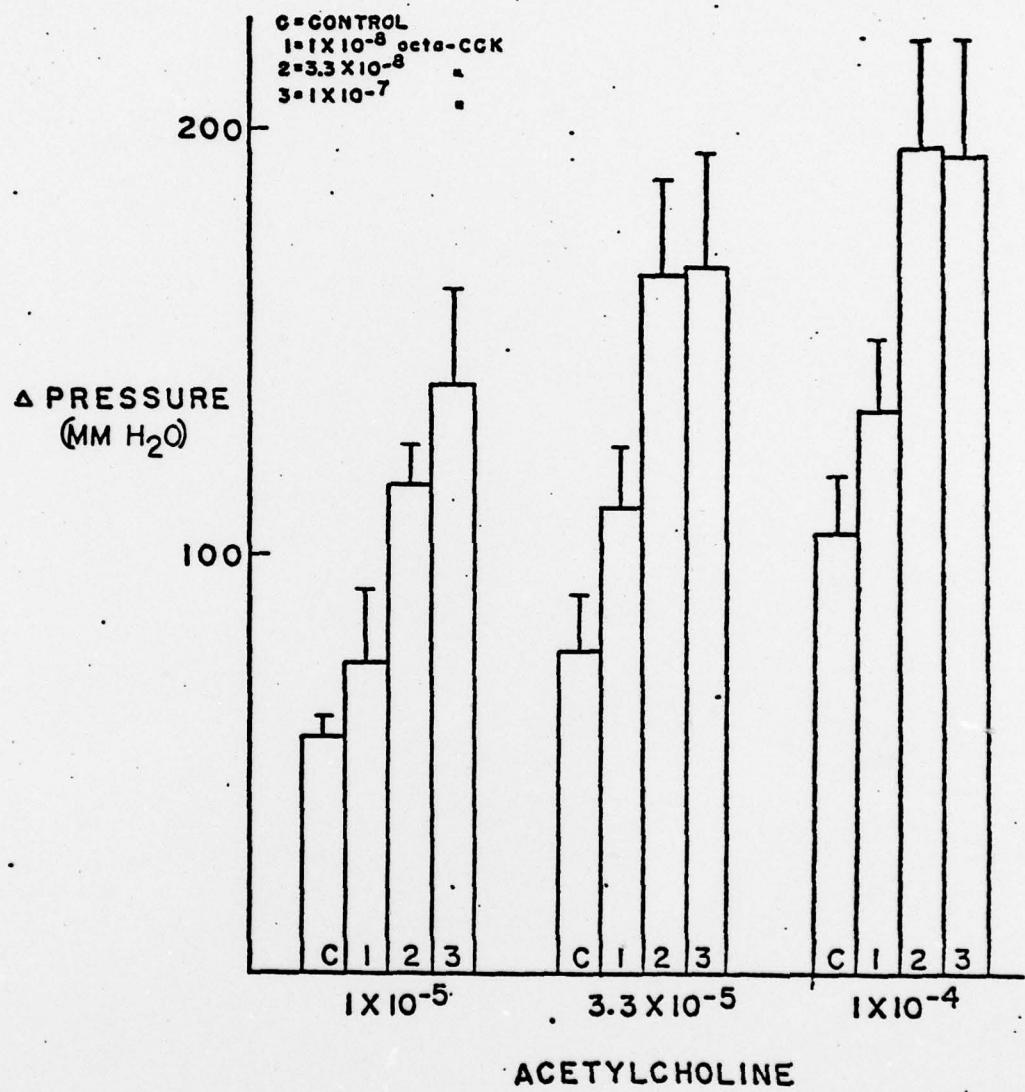
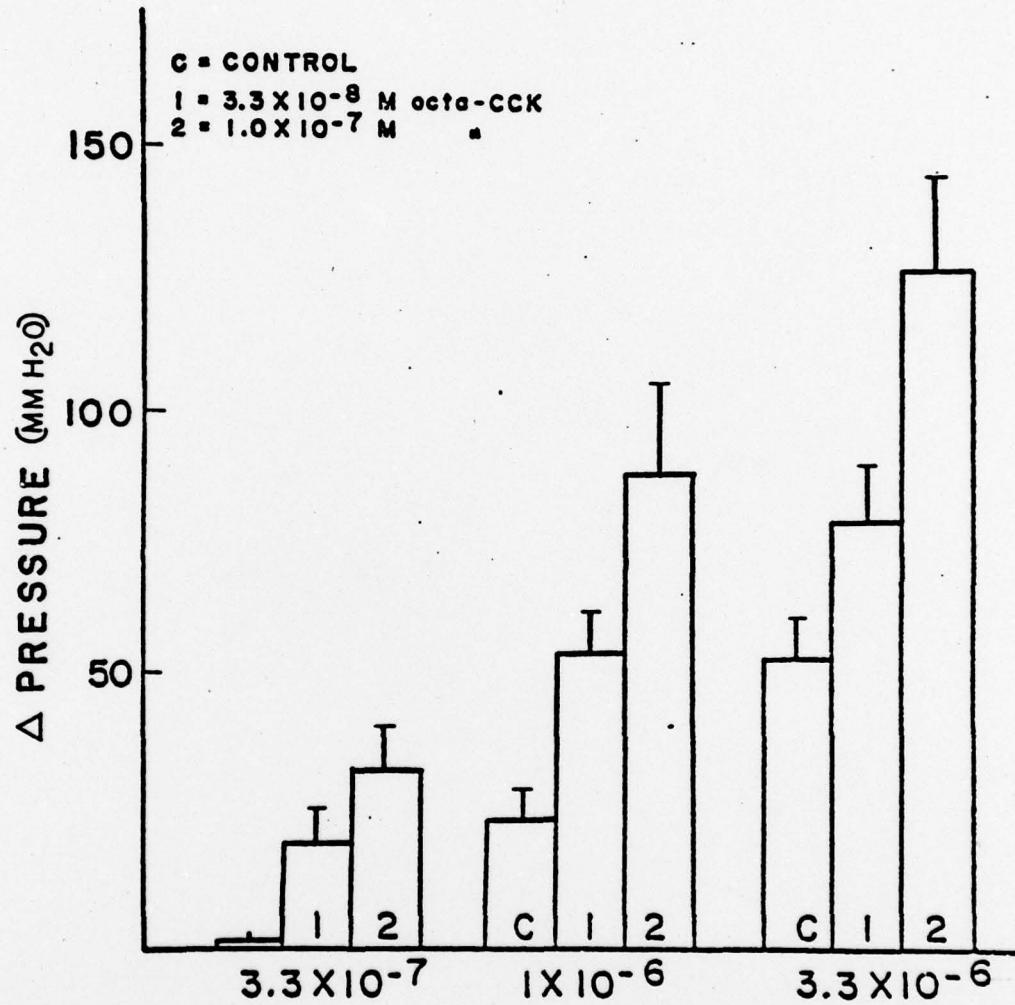


Figure 2. Potentiation by octa-CCK of the response of the guinea pig gall bladder to cumulative doses of acetylcholine. Each bar represents the mean (+ 1 S.E.M.) of three gall bladders. Pressure was measured in mm of water. Drug concentrations are reported as moles/liter.



OXOTREMORINE

Figure 3. Potentiation by octa-CCK of the response of the guinea pig gall bladder to cumulative doses of oxotremorine. Each bar represents the mean (\pm 1 S.E.M.) of three gall bladders. Pressure was measured in mm of water. Drug concentrations are reported as moles/liter.



the University of Alabama in Birmingham / UNIVERSITY STATION / BIRMINGHAM, ALABAMA 35294
the Medical Center / DEPARTMENT OF PHARMACOLOGY 1 March 1978

A STUDY OF A CONGENER OF PRIMAQUINE ON THE ISOLATED GALL BLADDER
AND ILEUM OF THE GUINEA PIG

ROBERT S. TEAGUE

ROY L. MUNDY

Contract Number

DADA 17-67-C-7136

Interim Report No. 13

ABSTRACT

The 6-hydroxy-8-aminoquinoline (WR 6890) congener of primaquine was studied in isolated smooth muscle preparations. The derivative neither contracted nor relaxed the isolated guinea pig ileum or gall bladder. The compound had no effect on the sensitivity of either tissue to cumulative doses of acetylcholine. The 6-methoxy-8-aminoquinoline derivative (Aldridge Chemical Co.) was insoluble in aqueous solvents and therefore could not be tested in the isolated organ preparations.

TO: Department of Pharmacology, Walter Reed Army Institute of Research,
Walter Reed Army Medical Center, Washington, D.C. 20012

SUBJECT: A Study of a Congener of Primaquine on the Isolated Gall Bladder
and Ileum of the Guinea Pig

INTRODUCTION

It has been shown that in the body 8-aminoquinoline antimalarial drugs can undergo both demethylation from the 6-methoxy position and side chain removal from the 8-amino position (1,2). The epigastric distress produced by primaquine ingestion might be produced by metabolites. In order to test this hypothesis, two putative metabolites were acquired and one of them studied for its effect on the isolated guinea pig gall bladder and ileum. One of the metabolites was not soluble in aqueous solvents and could not be tested on the biological preparations.

METHODS

Eighteen adult, male, Hartley guinea pigs were sacrificed by cervical dislocation. The gall bladder was quickly removed and placed in room temperature ($23 \pm 2^\circ\text{C}$) Tyrode's solution (3). Under low magnification the cystic duct was cut approximately two mm distal to the junction of the duct and the neck of the gall bladder. The duct was dilated with fine forceps and a half-inch long blunt, beveled, steel cannula (18 gauge hypodermic needle) inserted and tied in place. After removal of air bubbles by holding the needle upright and manual manipulation, the bladder was immersed in a chamber containing 20 ml of Tyrode's solution and the cannula attached to a 1280A Sanborn (Hewlett-Packard) pressure transducer (Serial No. DK) with a calibration factor of 15 MV/V.F.S. via a 30 cm length of Tyrode-filled tygon tubing (Intramedic (Clay-Adams) PE 20, ID 0.015 inches, OD 0.045 inches, which had been tested for animal experimentation). The transducer was energized and the signal amplified by a Sanborn 350-1100C (Hewlett-Packard) carrier preamplifier which was connected to a driver amplifier and power supply model 150-200B/400 (Hewlett-Packard) which supplied the signal to a galvanometer in the Hewlett-Packard, Series 7700, heat-writing recorder. The muscle chamber was maintained at 32°C in a ten gallon constant temperature bath (aquarium, Metaframe Corporation, 41 Slater Drive, Elmwood Park, NJ, 07407) (stirred and heated by a Thermomix 1420, B. Braun, Melsurger AG, Model IP 21, thermostatically controlled heating and stirring apparatus), and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The transducer and electrical recording system was calibrated by applying known pressures from a water manometer to the transducer.

A stopcock, 5 cm below the level of the bladder, was opened so that slight negative pressure emptied the gall bladder. The stopcock was closed and the bladder filled to a 100 μl volume. This volume produced an intraluminal pressure of approximately 100 mm of water (100 ± 15 mm). Drug-induced changes in pressure were measured as increases above this initial pressure.

Tissues were allowed to equilibrate in the chamber for thirty minutes during which time they were washed by overflowing 60 ml of fresh Tyrode's solution through the chamber two times.

A 3 cm length of ileum was suspended under 2 g resting tension in a 20 ml chamber containing Tyrode's solution (3). Drug-induced increases in tension were measured with a Hewlett-Packard force transducer FTA-10-1. The experiments were carried out by establishing a dose-response curve to cumulative additions of acetylcholine (10^{-7} , 3.3×10^{-7} , 10^{-6} M) in the absence and presence of 6-hydroxy-8-aminoquinoline. The 6-hydroxy-8-aminoquinoline was in contact with the muscle for 10 minutes prior to the lowest dose of acetylcholine being added and remained in the bath during the second and third cumulative additions of acetylcholine. The ileum was allowed to equilibrate in the muscle chamber for thirty minutes, in all experiments, before the commencement of the experimental protocol. During this equilibration period the tissue was washed two times with 60 ml of fresh Tyrode's solution.

The 6-hydroxy-8-aminoquinoline (WR 6890) was dissolved in deionized water and the pH of the resulting solution adjusted to approximately 9.5 with 1N sodium hydroxide. Injections of appropriate volumes of deionized water adjusted to pH 9.5 failed to alter the activity of the ileum or to change the pH of the bathing solution. The compound (WR 6890) appeared to remain in solution in the muscle chamber under the stated conditions.

The 6-methoxy-8-aminoquinoline (Aldridge Chemical Co.) was insoluble in deionized water. We were able to dissolve approximately 50 mg of the compound in a 1:1 mixture of propylene glycol and ethanol (2 ml), however the addition of water to the mixture caused precipitation of the chemical.

RESULTS

A concentration of 10^{-4} M 6-hydroxy-8-aminoquinoline did not contract or relax either the ileum or gall bladder in these experiments. There was no effect on the sensitivity of either tissue to acetylcholine. Table 1 summarizes the results obtained.

DISCUSSION

The 6-hydroxy-8-aminoquinoline (WR 6890) very probably has no role in the production of spasm or relaxation of the ileum or gall bladder after the oral injection of primaquine. The high concentration of the putative metabolite used in these studies makes it unlikely that concentrations in excess of the ones employed would ever be reached in an *in vivo* situation.

We were, of course, unable to study the 6-methoxy congener in our system. It is barely possible that this metabolite may be carried in biological systems in some solubilized form and the fact that we were not able to work with it does not mean that the compound is inactive.

Robert S. Teague
Robert S. Teague, M.D.
Principal Investigator

Roy L. Mundy
Roy L. Mundy, Ph.D.
Co-Investigator

REFERENCES

1. Baty, J. D., et al. Biomed. Mass Spectrom. 2:304-6, 1975.
2. Smith, C. C., J. Pharmacol. Expt. Therap. 116:67-76, 1956.
3. Staff of the Department of Pharmacology, University of Edinburgh: Pharmacological Experiments on Isolated Preparations. E. & S. Livingstone, Ltd., Edinburgh and London, 1968, p. 121.

TABLE I

The effect of 6-hydroxy-8-aminoquinoline (10^{-4} M) on the sensitivity of the guinea pig gall bladder and ileum to acetylcholine.

<u>GUINEA PIG GALL BLADDER</u>	<u>RESPONSE (mm H₂O)</u> Δ PRESSURE	
<u>Acetylcholine (Molar)</u>	<u>Control</u>	<u>6-OH-8-aminoquinoline (WR6890) Treated</u>
3.3×10^{-6}	$53.7 \pm 8.8^*$	46.7 ± 4.4
1.0×10^{-5}	98.0 ± 5.7	87.6 ± 3.9
3.3×10^{-5}	143.0 ± 10.6	136.3 ± 8.4

<u>GUINEA PIG ILEUM</u>	<u>RESPONSE (grams)</u> Δ TENSION	
<u>Acetylcholine (Molar)</u>	<u>Control</u>	<u>6-OH-8-aminoquinoline (WR 6890) Treated</u>
1.0×10^{-7}	$3.4 \pm 0.8^*$	4.6 ± 0.6
3.3×10^{-7}	4.7 ± 0.6	4.9 ± 1.4
1.0×10^{-6}	5.9 ± 1.1	6.2 ± 1.1

*Mean (+ S.E.M.) of 3 separate experiments on 3 strips of ileum and 3 gall bladders. No statistical treatment of the data was made because of the lack of any apparent reason for such treatment.

12 Copies

Director (ATTN: SGRD-UWZ-AG)
Walter Reed Army Institute of
Research
Walter Reed Army Medical Center
Washington, D. C. 20012

4 Copies

HQDA (SGRD-AJ)
Fort Detrick
Frederick, MD 21701

12 Copies

Defense Documentation Center
ATTN: DDC-TCA
Cameron Station
Alexandria, Virginia 22314

1 Copy

Dean
School of Medicine
Uniformed Services University of
the Health Sciences
4301 Jones Bridge Road
Bethesda, Maryland 20014

1 Copy

Superintendent
Academy of Health Science, US Army
ATTN: AHS-COM
Fort Sam Houston, Texas 78234